



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: C12N 15/55, A61K 38/46, C07K 16/40, C12N 9/20, C12N 15/63, C12Q 1/68	A2	(11) International Publication Number: WO 00/24911 (43) International Publication Date: 04 May 2000 (04.05.2000)
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(21) International Application Number: PCT/US99/25021

(22) International Filing Date: 27 October 1999 (27.10.1999)

Published

(30) Priority Data:

09/181,317 27 October 1998 (27.10.1998) US
09/234,726 21 January 1999 (21.01.1999) US

(60) Parent Application or Grant

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(54) Title: HUMAN PHOSPHOLIPASES

(54) Titre: PHOSPHOLIPASES HUMAINES

(57) Abstract

The invention provides human phospholipases (HPPL) and polynucleotides which identify and encode HPPL. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HPPL.

(57) Abrégé

L'invention concerne des phospholipases humaines (HPPL) et des polynucléotides qui identifient et codent les HPPL. L'invention concerne aussi des vecteurs d'expression, des cellules hôtes, des anticorps, des agonistes et des antagonistes. L'invention concerne enfin des procédés pour diagnostiquer, traiter ou prévenir les troubles liés à l'expression de HPPL.

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International Bureau



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		Published Without international search report and to be republished upon receipt of that report.	
(54) Title: HUMAN PHOSPHOLIPASES			
1 M E L A L L C G L V V M A - G V I P I Q G G I L N L N K M V 2641779 1 M K V L L L L A V V I M A F G S I Q V Q G S L L E F G Q M I GI 204319 30 K Q V T G K M P I L S Y W P Y G C H C G L G G R G Q P K D A 2641779 31 L F K T G K R A D V S Y G F Y G C H C G V G G R G S P K D A GI 204319 60 T D W C C Q T H D C C Y D H L K T Q G C G I Y K D Y Y R Y N 2641779 61 T D W C C V T H D C C Y N R L E K R G C G T K F L T Y K F S GI 204319 90 F S Q G N I H C S D K G S W C E Q Q L C A C D K E V A F C L 2641779 91 Y R G G Q I S C S T N Q D S C R K Q L C Q C D K A A A E C F GI 204319 120 K R N L D T Y Q K R L R F Y W R P H C R G Q T P G C 2641779 121 A R N K K S Y S L K Y Q F Y L N K F C K G K T P S C GI 204319			
(57) Abstract The invention provides human phospholipases (HPPL) and polynucleotides which identify and encode HPPL. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HPPL.			

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Description

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HUMAN PHOSPHOLIPASES

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of human phospholipases and to the use of these sequences in the diagnosis, treatment, and prevention of cancer, autoimmune/inflammatory disorders, and reproductive disorders.

BACKGROUND OF THE INVENTION

Triglyceride lipases are enzymes that hydrolyze the ester bond of triglycerides. Lipases are widely distributed in animals, plants, and prokaryotes. In higher vertebrates there are at least three tissue-specific isozymes including pancreatic, hepatic, and gastric lipases. These three types of lipases are structurally closely related to each other as well as to lipoprotein lipase. Gastric lipases in the intestine aid in the digestion and absorption of dietary fats. Adipocytes contain lipases that break down stored triacylglycerols, releasing fatty acids for export to other tissues where they are required as fuel. Gastric, hepatic, and pancreatic lipases hydrolyze lipoprotein triglycerides and phospholipids. The most conserved region in pancreatic, hepatic, and gastric lipases is centered around a serine residue which is also present in lipases of prokaryotic origin.

Phospholipases, a group of enzymes that catalyze the hydrolysis of membrane phospholipids, are classified according to the bond cleaved in a phospholipid. They are classified into PLA1, PLA2, PLB, PLC, and PLD. Phospholipases are involved in many inflammatory reactions by making arachidonate available for eicosanoid biosynthesis. More specifically, arachidonic acid is processed into bioactive lipid mediators of inflammation such as lyso-platelet-activating factor and eicosanoids. The synthesis of arachidonic acid from membrane phospholipids is the rate-limiting step in the biosynthesis of the four major classes of eicosanoids which are prostaglandins, prostacyclins, thromboxanes and leukotrienes, and are involved in pain, fever, and inflammation. (Kaiser, E. et al. (1990) Clin. Biochem. 23:349-370.) Furthermore, leukotriene-B4 is known to function in a feedback loop which further increases PLA2 activity. (Wijkander, J. et al. (1995) J. Biol. Chem. 270:26543-26549.)

The secretory phospholipase A₂ (PLA2) superfamily comprises a number of heterogeneous enzymes whose common feature is to hydrolyze the *sn*-2 fatty acid acyl ester bond of phosphoglycerides. Hydrolysis of the glycerophospholipids releases free fatty acids and lysophospholipids. PLA2 activity generates precursors for the biosynthesis of biologically active lipids, hydroxy fatty acids, and platelet-activating factor.

PLA2s were first described as components of snake venoms, and were later characterized in numerous species. PLA2s have traditionally been classified into several major groups and subgroups

5 based on their amino acid sequences, divalent cation requirements, and location of disulfide bonds. The PLA2s of Groups I, II, and III consist of low molecular weight, secreted, Ca^{2+} -dependent proteins. Group IV PLA2s are primarily 85-kDa, Ca^{2+} -dependent cytosolic phospholipases. Finally, a number of Ca^{2+} -independent PLA2s have been described, which comprise Group V. (Davidson, F. F. and Dennis, E. A., (1990) *J. Mol. Evol.* 31: 228-238; and Dennis, E. F. (1994) *J. Biol. Chem.* 269:13057-13060.)

15 The first PLA2s to be extensively characterized were the Group I, II, and III PLA2s found in snake and bee venoms. These venom PLA2s share many features with mammalian PLA2s including a common catalytic mechanism, the same Ca^{2+} requirement, and conserved primary and tertiary structures. In addition to their role in the digestion of prey, the venom PLA2s display neurotoxic, myotoxic, anticoagulant, and proinflammatory effects in mammalian tissues. This diversity of pathophysiological effects is due to the presence of specific, high affinity receptors for these enzymes on various cells and tissues. (Lambeau, G. et al. (1995) *J. Biol. Chem.* 270:5534-5540.)

20 PLA2s from Groups I, IIA, IIC, and V have been described in mammalian and avian cells, and were originally characterized by tissue distribution, although the distinction is no longer absolute. Thus, Group I PLA2s were found in the pancreas, Group IIA and IIC derived from inflammation-associated tissues (e.g., the synovium), and Group V were from cardiac tissue. The pancreatic PLA2s function in the digestion of dietary lipids and have been proposed to play a role in cell proliferation, smooth muscle contraction, and acute lung injury. The inflammatory PLA2s are potent mediators of inflammatory processes and are highly expressed in serum and synovial fluids of patients with inflammatory disorders. These Group II PLA2s are found in most human cell types assayed and are expressed in diverse pathological processes such as septic shock, intestinal cancers, rheumatoid arthritis, and epidermal hyperplasia. A Group V PLA2 has been cloned from brain tissue and is strongly expressed in heart tissue. A human PLA2 was recently cloned from fetal lung, and based on its structural properties, appears to be the first member of a new group of mammalian PLAs, referred to as Group X. Other PLA2s have been cloned from various human tissues and cell lines, suggesting a large diversity of PLA2s. (Chen, J. et al. (1994) *J. Biol. Chem.* 269:2365-2368; Kennedy, B.P., et al. (1995) *J. Biol. Chem.* 270: 22378-22385; Komada, M., et al. (1990) *Biochem. Biophys. Res. Commun.* 168: 1059-1065; Cupillard, L. et al. (1997) *J. Biol. Chem.* 272:15745-15752; and Nalefski, E.A. et al. (1994) *J. Biol. Chem.* 269:18239-18249.)

45 Rat platelets secrete two types of phospholipases when stimulated, type II phospholipase A2 and phosphatidylserine-phospholipase A1 (PS-PLA1). PS-PLA1, also referred to as serine-phospholipid-selective phospholipase A (PLA), was purified from rat platelets and found to be a 55-kDa protein with active serine residues. The predicted 456-amino acid sequence contains a putative short N-terminal signal sequence and a GX SXG motif (SEQ ID NO:10). PS-PLA1 shares about 30%

homology with mammalian lipases such as lipoprotein lipase, hepatic lipase, and pancreatic lipase. The residues that surround the active serine in PS-PLA1 include histidine and aspartic acid. The serine, histidine, and aspartic acid residues form a triad that is highly conserved among mammalian lipases. PS-PLA1 hydrolyzes a fatty acyl residue at the sn-1 position of lysophosphatidylserine and phosphatidylserine, and appears to be the first phospholipase that exclusively hydrolyzes the sn-1 position. (Sato, T. et al. (1997) J. Biol. Chem. 272:2192-2198.)

PLAs are implicated in a variety of disease processes. For example, PLAs are found in the pancreas, in cardiac tissue, and in inflammation-associated tissues. Pancreatic PLAs function in the digestion of dietary lipids and have been proposed to play a role in cell proliferation, smooth muscle contraction, and acute lung injury. Inflammatory PLAs are potent mediators of inflammatory processes and are highly expressed in serum and synovial fluids of patients with inflammatory disorders. Additionally, inflammatory PLAs are found in most human cell types and are expressed in diverse pathological processes such as septic shock, intestinal cancers, rheumatoid arthritis, and epidermal hyperplasia.

The discovery of new human phospholipases and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cancer, autoimmune/inflammatory disorders, and reproductive disorders.

SUMMARY OF THE INVENTION

The invention features substantially purified polypeptides, human phospholipases, referred to collectively as "HPPL" and individually as "HPPL-1," "HPPL-2," and "HPPL-3." In one aspect, the invention provides a substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof. The invention also includes a polypeptide comprising an amino acid sequence that differs by one or more conservative amino acid substitutions from an amino acid sequence selected from the group consisting of SEQ ID NO:1-3.

The invention further provides a substantially purified variant having at least 90% amino acid identity to at least one of the amino acid sequences selected from the group consisting of SEQ ID NO:1-3 and fragments thereof. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid

5 sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof.

10 5 The invention also provides a method for detecting a polynucleotide in a sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide sequence to at least one of the polynucleotides of the sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide in the sample. In one aspect, 15 the method further comprises amplifying the polynucleotide prior to hybridization.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:4-6 and fragments thereof. 20 The invention further provides an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide sequence selected from the group consisting of SEQ ID NO:4-6 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:4-6 and fragments thereof. 25

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-3. In another aspect, the expression vector is contained within a host cell. 30

The invention also provides a method for producing a polypeptide, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing a polynucleotide of the invention under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture. 35

25 The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

40 The invention further includes a purified antibody which binds to a polypeptide selected from the group consisting of SEQ ID NO:1-3 and fragments thereof. The invention also provides a purified agonist and a purified antagonist to the polypeptide. 30

45 The invention also provides a method for treating or preventing a disorder associated with decreased expression or activity of HPPL, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof, in conjunction with a suitable pharmaceutical carrier. 50

The invention also provides a method for treating or preventing a disorder associated with increased expression or activity of HPPL, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof.

BRIEF DESCRIPTION OF THE FIGURES AND TABLES

Figure 1 shows the amino acid sequence alignment between HPPL-1 (Incyte Clone number 2641779; SEQ ID NO:1) and group II phospholipase A-2 (GI 204319; SEQ ID NO:7), produced using the multisequence alignment program of LASERGENE software (DNASTAR, Madison WI).

Figures 2A, 2B, and 2C show the amino acid sequence alignment between HPPL-2 (Incyte Clone number 1430683; SEQ ID NO:2) and cytosolic phospholipase A2 (GI 508625; SEQ ID NO:8).

Figures 3A and 3B show the amino acid sequence alignment between HPPL-3 (Incyte Clone number 1316804; SEQ ID NO:3) and rat phosphatidylserine-phospholipase A1 (PS-PLA1) (GI 1817556; SEQ ID NO:9).

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HPPL.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of HPPL.

Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding HPPL were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze HPPL, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

5 It must be noted that as used herein and in the appended claims, the singular forms "a," "an,"
and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a
reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a
reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so
10 5 forth.

Unless defined otherwise, all technical and scientific terms used herein have the same
meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.
Although any machines, materials, and methods similar or equivalent to those described herein can be
15 used to practice or test the present invention, the preferred machines, materials and methods are now
described. All publications mentioned herein are cited for the purpose of describing and disclosing
the cell lines, protocols, reagents and vectors which are reported in the publications and which might
be used in connection with the invention. Nothing herein is to be construed as an admission that the
invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

15 "HPPL" refers to the amino acid sequences of substantially purified HPPL obtained from any
species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and
human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of
HPPL. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other
20 compound or composition which modulates the activity of HPPL either by directly interacting with
HPPL or by acting on components of the biological pathway in which HPPL participates.

An "allelic variant" is an alternative form of the gene encoding HPPL. Allelic variants may
result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in
polypeptides whose structure or function may or may not be altered. A gene may have none, one, or
35 many allelic variants of its naturally occurring form. Common mutational changes which give rise to
allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides.
Each of these types of changes may occur alone, or in combination with the others, one or more times
in a given sequence.

"Altered" nucleic acid sequences encoding HPPL include those sequences with deletions,
30 insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as HPPL or a
polypeptide with at least one functional characteristic of HPPL. Included within this definition are
polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe
of the polynucleotide encoding HPPL, and improper or unexpected hybridization to allelic variants,
with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding
45 HPPL. The encoded protein may also be "altered," and may contain deletions, insertions, or
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5 substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HPPL. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HPPL is retained. For example,

10 5 negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

15 10 The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

20 15 "Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

25 30 The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of HPPL. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HPPL either by directly interacting with HPPL or by acting on components of the biological pathway in which HPPL participates.

35 35 The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind HPPL polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

40 45 The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures

5 on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

10 The term "antisense" refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand.

15 The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HPPL, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

20 The terms "complementary" and "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acid strands, and in the design and use of peptide nucleic acid (PNA) molecules.

35 A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution.

25 Compositions comprising polynucleotide sequences encoding HPPL or fragments of HPPL may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

40 "Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of one or more Incyte Clones and, in some cases, one or more public domain ESTs, using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

Original Residue	Conservative Substitution
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser
Gln	Asn, Glu, His
Glu	Asp, Gln, His
Gly	Ala
His	Asn, Arg, Gln, Glu
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	His, Met, Leu, Trp, Tyr
Ser	Cys, Thr
Thr	Ser, Val
Trp	Phe, Tyr
Tyr	His, Phe, Trp
Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "fragment" is a unique portion of HPPL or the polynucleotide encoding HPPL which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up

5 to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a
fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment
used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15,
10 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues
5 in length. Fragments may be preferentially selected from certain regions of a molecule. For example,
a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the
first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined
sequence. Clearly these lengths are exemplary, and any length that is supported by the specification,
15 including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

10 A fragment of SEQ ID NO:4-6 comprises a region of unique polynucleotide sequence that
specifically identifies SEQ ID NO:4-6, for example, as distinct from any other sequence in the same
genome. A fragment of SEQ ID NO:4-6 is useful, for example, in hybridization and amplification
20 technologies and in analogous methods that distinguish SEQ ID NO:4-6 from related polynucleotide
sequences. The precise length of a fragment of SEQ ID NO:4-6 and the region of SEQ ID NO:4-6 to
15 which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on
the intended purpose for the fragment.

25 A fragment of SEQ ID NO:1-3 is encoded by a fragment of SEQ ID NO:4-6. A fragment of
SEQ ID NO:1-3 comprises a region of unique amino acid sequence that specifically identifies SEQ ID
NO:1-3. For example, a fragment of SEQ ID NO:1-3 is useful as an immunogenic peptide for the
30 20 development of antibodies that specifically recognize SEQ ID NO:1-3. The precise length of a
fragment of SEQ ID NO:1-3 and the region of SEQ ID NO:1-3 to which the fragment corresponds are
routinely determinable by one of ordinary skill in the art based on the intended purpose for the
fragment.

35 The term "similarity" refers to a degree of complementarity. There may be partial similarity
25 or complete similarity. The word "identity" may substitute for the word "similarity." A partially
complementary sequence that at least partially inhibits an identical sequence from hybridizing to a
target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the
40 completely complementary sequence to the target sequence may be examined using a hybridization
assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced
30 stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the
binding of a completely similar (identical) sequence to the target sequence under conditions of
45 reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific
binding is permitted, as reduced stringency conditions require that the binding of two sequences to
one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be
50 35 tested by the use of a second target sequence which lacks even a partial degree of complementarity

5 (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

10 The phrases "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

15 Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 20 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequence pairs.

25 Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 30 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

45 *Matrix: BLOSUM62*

Reward for match: 1

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

50 *Gap x drop-off: 50*

Expect: 10

Word Size: 11

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Open Gap: 11 and Extension Gap: 1 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention

5 include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, 10 5 denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be 15 suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides. 10

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A 20 hybridization complex may be formed in solution (e.g., C₀t or R₀t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate 15 to which cells or their nucleic acids have been fixed). 25

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune 30 disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems. 20

The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate.

The terms "element" and "array element" in a microarray context, refer to hybridizable 35 polynucleotides arranged on the surface of a substrate. 25

The term "modulate" refers to a change in the activity of HPPL. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other 40 biological, functional, or immunological properties of HPPL. 40

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, 30 polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. 45

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably 50 linked to a coding sequence if the promoter affects the transcription or expression of the coding 35

5 sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

10 "Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

15 "Probe" refers to nucleic acid sequences encoding HPPL, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

20 "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

25 Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

30 Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel et al., 1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

35 Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase

5 sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer
selection program (available to the public from the Whitehead Institute/MIT Center for Genome
Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to
10 avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of
oligonucleotides for microarrays. (The source code for the latter two primer selection programs may
also be obtained from their respective sources and modified to meet the user's specific needs.) The
PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource
Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing
15 selection of primers that hybridize to either the most conserved or least conserved regions of aligned
nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved
oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments
identified by any of the above selection methods are useful in hybridization technologies, for
20 example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or
partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide
selection are not limited to those described above.

25 A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence
that is made by an artificial combination of two or more otherwise separated segments of sequence.
This artificial combination is often accomplished by chemical synthesis or, more commonly, by the
artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques
30 such as those described in Sambrook, *supra*. The term recombinant includes nucleic acids that have
been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a
recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter
sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to
35 transform a cell.

25 Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a
vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is
expressed, inducing a protective immunological response in the mammal.

40 The term "sample" is used in its broadest sense. A sample suspected of containing nucleic
acids encoding HPPL, or fragments thereof, or HPPL itself, may comprise a bodily fluid; an extract
30 from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or
cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

45 The terms "specific binding" and "specifically binding" refer to that interaction between a
protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or
synthetic binding composition. The interaction is dependent upon the presence of a particular
35 structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding

5 molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

10 The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

15 A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

20 "Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

25 "Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells
30 includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

35 A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or
40 greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may
30 have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to
45 each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene
50

5 between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

10 5 A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at
15 at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence
10 identity over a certain defined length of one of the polypeptides.

20 THE INVENTION

The invention is based on the discovery of new human phospholipases (HPPL), the polynucleotides encoding HPPL, and the use of these compositions for the diagnosis, treatment, or
15 prevention of cancer, autoimmune/inflammatory disorders, and reproductive disorders.

25 Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding HPPL. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which
30 nucleic acids encoding each HPPL were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA
20 libraries. The Incyte clones in column 5 were used to assemble the consensus nucleotide sequence of each HPPL and are useful as fragments in hybridization technologies.

35 The columns of Table 2 show various properties of each of the polypeptides of the invention:
25 column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6
40 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The
30 methods of column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

45 HPPL-1 has chemical and structural similarity with group II phospholipase A-2 from rat (GI 204319; SEQ ID NO:7). In particular, HPPL-1 and group II phospholipase A-2 share 46% identity. HPPL-2 has chemical and structural similarity with cytosolic phospholipase A2 from chicken (GI
50 508625; SEQ ID NO:8). In particular, HPPL-2 and cytosolic phospholipase A2 share 20% identity.

5 HPPL-3 has chemical and structural similarity with PS-PLA1 (GI 1817556; SEQ ID NO:9). In particular, HPPL-3 and PS-PLA1 share 80% identity. HPPL-3 and PS-PLA1 each contain a short N-terminal signal sequence, share the conserved potential lipase serine active site at residue I160 and the GX SXG (SEQ ID NO:10) consensus pattern.

10 5 The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding HPPL. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:4-6 and to distinguish between SEQ ID NO:4-6 and related polynucleotide sequences. The polypeptides
15 encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express HPPL as a fraction of total tissues expressing HPPL. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing HPPL as a fraction of total tissues expressing HPPL. Column 5 lists the vectors used to subclone each cDNA library. Of
20 particular note is the expression of HPPL-1 (SEQ ID NO:1) in prostate, breast, and testicular tumors, the expression of HPPL-2 (SEQ ID NO:2) in tumors of the ovary, breast, and brain, and the
25 expression of HPPL-3 (SEQ ID NO:3) in gastrointestinal and reproductive tissue.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding HPPL were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows
30 the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

The invention also encompasses HPPL variants. A preferred HPPL variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the HPPL amino acid sequence, and which contains at least one functional or structural characteristic of HPPL.

35 25 The invention also encompasses polynucleotides which encode HPPL. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:4-6, which encodes HPPL.

40 The invention also encompasses a variant of a polynucleotide sequence encoding HPPL. In particular, such a variant polynucleotide sequence will have at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HPPL. A particular aspect of the invention encompasses a variant of a
45 polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:4-6 which has at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of
50 35 SEQ ID NO:4-6. Any one of the polynucleotide variants described above can encode an amino acid

5 sequence which contains at least one functional or structural characteristic of HPPL.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HPPL, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be
10 5 produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HPPL, and all such variations are to be considered as
15 being specifically disclosed.

10 Although nucleotide sequences which encode HPPL and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring HPPL under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HPPL or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide
20 occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HPPL and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

20 The invention also encompasses production of DNA sequences which encode HPPL and HPPL derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce
35 mutations into a sequence encoding HPPL or any fragment thereof.

25 Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:4-6 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in
30 "Definitions."

45 Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or
50 35 combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE

5 amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is
automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV),
PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler
10 (Perkin-Elmer). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing
5 system (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics,
Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a
variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short
Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995)
15 Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

10 The nucleic acid sequences encoding HPPL may be extended utilizing a partial nucleotide
sequence and employing various PCR-based methods known in the art to detect upstream sequences,
such as promoters and regulatory elements. For example, one method which may be employed,
20 restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic
DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.)
15 Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown
sequence from a circularized template. The template is derived from restriction fragments comprising
a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids
Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent
25 to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al.
20 (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and
ligations may be used to insert an engineered double-stranded sequence into a region of unknown
sequence before performing PCR. Other methods which may be used to retrieve unknown sequences
are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060).
35 Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo
25 Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in
finding intron/exon junctions. For all PCR-based methods, primers may be designed using
commercially available software, such as OLIGO 4.06 Primer Analysis software (National
40 Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in
length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of
30 about 68°C to 72°C.

45 When screening for full-length cDNAs, it is preferable to use libraries that have been
size-selected to include larger cDNAs. In addition, random-primed libraries, which often include
sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T)
library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence
50 into 5' non-transcribed regulatory regions.

5 Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the
10 5 emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be
15 present in limited amounts in a particular sample.

10 In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HPPL may be cloned in recombinant DNA molecules that direct expression of HPPL, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent
20 degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HPPL.

15 The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HPPL-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA
25 shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction
30 sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding HPPL may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids
35 Symp. Ser. 7:215-223; and Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.)

25 Alternatively, HPPL itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the
40 ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HPPL, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other
30 proteins, or any part thereof, to produce a variant polypeptide.

45 The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New
50 35 York NY.)

5 In order to express a biologically active HPPL, the nucleotide sequences encoding HPPL or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and
10 5 inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding HPPL. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HPPL. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where
15 sequences encoding HPPL and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be
20 provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of
15 enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

25 Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HPPL and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning. A Laboratory
30 Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

35 A variety of expression vector/host systems may be utilized to contain and express sequences encoding HPPL. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with
25 yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or
40 animal cell systems. The invention is not limited by the host cell employed.

30 In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HPPL. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HPPL can be achieved using a
45 multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding HPPL into the vector's multiple
50 cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of

transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HPPL are needed, e.g. for the production of antibodies, vectors which direct high level expression of HPPL may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HPPL. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of HPPL. Transcription of sequences encoding HPPL may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HPPL may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HPPL in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

5 For long term production of recombinant proteins in mammalian systems, stable expression of
HPPL in cell lines is preferred. For example, sequences encoding HPPL can be transformed into cell
lines using expression vectors which may contain viral origins of replication and/or endogenous
10 expression elements and a selectable marker gene on the same or on a separate vector. Following the
introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media
before being switched to selective media. The purpose of the selectable marker is to confer resistance
to a selective agent, and its presence allows growth and recovery of cells which successfully express
the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue
15 culture techniques appropriate to the cell type.

10 Any number of selection systems may be used to recover transformed cell lines. These
include, but are not limited to, the herpes simplex virus thymidine kinase and adenine
phosphoribosyltransferase genes, for use in *tk* and *ap^r* cells, respectively. (See, e.g., Wigler, M. et
al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic,
20 or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to
methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat*
confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g.,
Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981)
J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which
25 alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc.
Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins
(GFP; Clontech), β glucuronidase and its substrate β -glucuronide, or luciferase and its substrate
luciferin may be used. These markers can be used not only to identify transformants, but also to
quantify the amount of transient or stable protein expression attributable to a specific vector system.
30 (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

25 Although the presence/absence of marker gene expression suggests that the gene of interest is
also present, the presence and expression of the gene may need to be confirmed. For example, if the
sequence encoding HPPL is inserted within a marker gene sequence, transformed cells containing
sequences encoding HPPL can be identified by the absence of marker gene function. Alternatively, a
marker gene can be placed in tandem with a sequence encoding HPPL under the control of a single
30 promoter. Expression of the marker gene in response to induction or selection usually indicates
expression of the tandem gene as well.

45 In general, host cells that contain the nucleic acid sequence encoding HPPL and that express
HPPL may be identified by a variety of procedures known to those of skill in the art. These
procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR
35 amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or

chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HPPL using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HPPL is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HPPL include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HPPL, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HPPL may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HPPL may be designed to contain signal sequences which direct secretion of HPPL through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity.

Different host cells which have specific cellular machinery and characteristic mechanisms for

5 post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

10 In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HPPL may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HPPL protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HPPL activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HPPL encoding sequence and the heterologous protein sequence, so that HPPL may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10).
20 A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

35 In a further embodiment of the invention, synthesis of radiolabeled HPPL may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ³⁵S-methionine.

40 Fragments of HPPL may be produced not only by recombinant means, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra, pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A peptide synthesizer (Perkin-Elmer). Various fragments of HPPL may be synthesized separately and then combined to produce the full length molecule.

45 THERAPEUTICS

50 Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of HPPL and human phospholipases. In addition, the expression of HPPL is closely associated with cancerous, inflammation and the immune response, and with gastrointestinal and

5 reproductive tissues. Therefore, HPPL appears to play a role in cancer, autoimmune/inflammatory disorders, and reproductive disorders. In the treatment of disorders associated with increased HPPL expression or activity, it is desirable to decrease the expression or activity of HPPL. In the treatment of disorders associated with decreased HPPL expression or activity, it is desirable to increase the
10 expression or activity of HPPL.

Therefore, in one embodiment, HPPL or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HPPL. Examples of such disorders include, but are not limited to, a cancer such as adenocarcinoma,
15 leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia,
20 gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an autoimmune/inflammatory disorder, such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis,
25 autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable
30 bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal,
35 parasitic, protozoal, and helminthic infections, and trauma; and a reproductive disorder, such as disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast
40 disease, and galactorrhœa; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia.

In another embodiment, a vector capable of expressing HPPL or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased
50 expression or activity of HPPL including, but not limited to, those described above.

5 In a further embodiment, a pharmaceutical composition comprising a substantially purified HPPL in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HPPL including, but not limited to, those provided above.

10 5 In still another embodiment, an agonist which modulates the activity of HPPL may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HPPL including, but not limited to, those listed above.

15 In a further embodiment, an antagonist of HPPL may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HPPL. Examples of such disorders include, but are not limited to, those cancer, autoimmune/inflammatory disorders, and reproductive disorders described above. In one aspect, an antibody which specifically binds HPPL may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HPPL.

20 In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HPPL may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HPPL including, but not limited to, those described above.

25 In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

30 An antagonist of HPPL may be produced using methods which are generally known in the art. In particular, purified HPPL may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HPPL. Antibodies to HPPL may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

35 For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HPPL or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols,

5 polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

10 It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HPPL have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HPPL amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

15 Monoclonal antibodies to HPPL may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

25 In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HPPL-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

30 Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

35 Antibody fragments which contain specific binding sites for HPPL may also be generated. For example, such fragments include, but are not limited to, F(ab')₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

40 Various immunoassays may be used for screening to identify antibodies having the desired

5 specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HPPL and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies
10 5 reactive to two non-interfering HPPL epitopes is generally used, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for HPPL. Affinity is expressed as an association constant, K_a , which is defined as the molar concentration of HPPL-antibody complex
15 10 divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple HPPL epitopes, represents the average affinity, or avidity, of the antibodies for HPPL. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular HPPL epitope, represents a true measure of affinity. High-affinity antibody preparations
20 15 with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the HPPL-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of HPPL, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington, DC;
25 30 Liddell, J.E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably
35 25 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of HPPL-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)
40

In another embodiment of the invention, the polynucleotides encoding HPPL, or any fragment
30 45 or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding HPPL may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HPPL. Thus, complementary molecules or fragments may be used to modulate HPPL activity, or to achieve regulation of gene function. Such technology is now well
50 35 known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from

5 various locations along the coding or control regions of sequences encoding HPPL.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HPPL. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

10 Genes encoding HPPL can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding HPPL. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding HPPL. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may be employed. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

25 Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HPPL.

30 Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary

oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis.

Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding HPPL. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use *in vivo*, *in vitro*, and *ex vivo*. For *ex vivo* therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient.

Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HPPL, antibodies to HPPL, and mimetics, agonists, antagonists, or inhibitors of HPPL. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial,

5 intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

10 In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

15 Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration.

20 Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

25 Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

30 Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

35 Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

40 Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or

5 dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily
injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or
synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic
10 amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable
5 stabilizers or agents to increase the solubility of the compounds and allow for the preparation of
highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be
permeated are used in the formulation. Such penetrants are generally known in the art.

15 The pharmaceutical compositions of the present invention may be manufactured in a manner
that is known in the art, e.g., by means of conventional mixing, dissolving, granulating,
10 dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

20 The pharmaceutical composition may be provided as a salt and can be formed with many
acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic
acids. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding
15 free base forms. In other cases, the preparation may be a lyophilized powder which may contain any
or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a
25 pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate
container and labeled for treatment of an indicated condition. For administration of HPPL, such
30 labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein
the active ingredients are contained in an effective amount to achieve the intended purpose. The
determination of an effective dose is well within the capability of those skilled in the art.

35 For any compound, the therapeutically effective dose can be estimated initially either in cell
25 culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, or pigs.
An animal model may also be used to determine the appropriate concentration range and route of
administration. Such information can then be used to determine useful doses and routes for
40 administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HPPL
30 or fragments thereof, antibodies of HPPL, and agonists, antagonists or inhibitors of HPPL, which
ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by
45 standard pharmaceutical procedures in cell cultures or with experimental animals, such as by
calculating the ED₅₀ (the dose therapeutically effective in 50% of the population) or LD₅₀ (the dose
lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the
50 therapeutic index, which can be expressed as the LD₅₀/ED₅₀ ratio. Pharmaceutical compositions

5 which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and
animal studies are used to formulate a range of dosage for human use. The dosage contained in such
compositions is preferably within a range of circulating concentrations that includes the ED₅₀ with
10 little or no toxicity. The dosage varies within this range depending upon the dosage form employed,
the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the
subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the
active moiety or to maintain the desired effect. Factors which may be taken into account include the
15 severity of the disease state, the general health of the subject, the age, weight, and gender of the
subject, time and frequency of administration, drug combination(s), reaction sensitivities, and
response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4
days, every week, or biweekly depending on the half-life and clearance rate of the particular
20 formulation.

Normal dosage amounts may vary from about 0.1 μ g to 100,000 μ g, up to a total dose of
15 about 1 gram, depending upon the route of administration. Guidance as to particular dosages and
methods of delivery is provided in the literature and generally available to practitioners in the art.
Those skilled in the art will employ different formulations for nucleotides than for proteins or their
25 inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells,
conditions, locations, etc.

30 DIAGNOSTICS

In another embodiment, antibodies which specifically bind HPPL may be used for the
diagnosis of disorders characterized by expression of HPPL, or in assays to monitor patients being
treated with HPPL or agonists, antagonists, or inhibitors of HPPL. Antibodies useful for diagnostic
35 purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays
for HPPL include methods which utilize the antibody and a label to detect HPPL in human body fluids
or in extracts of cells or tissues. The antibodies may be used with or without modification, and may
be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter
40 molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring HPPL, including ELISAs, RIAs, and FACS, are known
30 in the art and provide a basis for diagnosing altered or abnormal levels of HPPL expression. Normal
or standard values for HPPL expression are established by combining body fluids or cell extracts
taken from normal mammalian subjects, for example, human subjects, with antibody to HPPL under
conditions suitable for complex formation. The amount of standard complex formation may be
45 quantitated by various methods, such as photometric means. Quantities of HPPL expressed in subject,
control, and disease samples from biopsied tissues are compared with the standard values. Deviation
50

5 between standard and subject values establishes the parameters for diagnosing disease.

10 In another embodiment of the invention, the polynucleotides encoding HPPL may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of HPPL may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HPPL, and to monitor regulation of HPPL levels during therapeutic intervention.

15 In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HPPL or closely related molecules may be used to identify nucleic acid sequences which encode HPPL. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding HPPL, allelic variants, or related sequences.

20 Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the HPPL encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:4-6 or from genomic sequences including promoters, enhancers, and introns of the HPPL gene.

25 Means for producing specific hybridization probes for DNAs encoding HPPL include the cloning of polynucleotide sequences encoding HPPL or HPPL derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ³²P or ³⁵S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

30 Polynucleotide sequences encoding HPPL may be used for the diagnosis of disorders associated with expression of HPPL. Examples of such disorders include, but are not limited to, a cancer such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an autoimmune/inflammatory disorder, such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact

dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and a reproductive disorder, such as disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia. The polynucleotide sequences encoding HPPL may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered HPPL expression.

Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HPPL may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HPPL may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HPPL in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HPPL, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HPPL, under conditions suitable for hybridization or amplification.

Standard hybridization may be quantified by comparing the values obtained from normal subjects

5 with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

10 5 Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

15 10 With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

25 Additional diagnostic uses for oligonucleotides designed from the sequences encoding HPPL may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced *in vitro*. Oligomers will preferably contain a fragment of a polynucleotide encoding HPPL, or a fragment of a polynucleotide complementary to the polynucleotide encoding HPPL, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

35 Methods which may also be used to quantify the expression of HPPL include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

40 30 In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

5 Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g.,
Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci.
USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al.
10 (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-
2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HPPL may be used
to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The
sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to
15 artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial
chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single
10 chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price,
C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

20 Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome
mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra,
pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the
15 Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the
25 location of the gene encoding HPPL on a physical chromosomal map and a specific disorder, or a
predisposition to a specific disorder, may help define the region of DNA associated with that disorder.
The nucleotide sequences of the invention may be used to detect differences in gene sequences among
20 normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as
linkage analysis using established chromosomal markers, may be used for extending genetic maps.
Often the placement of a gene on the chromosome of another mammalian species, such as mouse,
35 may reveal associated markers even if the number or arm of a particular human chromosome is not
25 known. New sequences can be assigned to chromosomal arms by physical mapping. This provides
valuable information to investigators searching for disease genes using positional cloning or other
gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic
40 linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping
to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti,
30 R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be
used to detect differences in the chromosomal location due to translocation, inversion, etc., among
45 normal, carrier, or affected individuals.

In another embodiment of the invention, HPPL, its catalytic or immunogenic fragments, or
oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug
50 35 screening techniques. The fragment employed in such screening may be free in solution, affixed to a

5 solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HPPL and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with HPPL, or fragments thereof, and washed. Bound HPPL is then detected by methods well known in the art. Purified HPPL can also be coated directly onto plates for use in the aforementioned drug screening techniques.

10 Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HPPL specifically compete with a test compound for binding HPPL. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HPPL.

15 In additional embodiments, the nucleotide sequences which encode HPPL may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

20 Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

30 Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

40 The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. [Attorney Docket No. PF-0625 P, filed October 27, 1998] and U.S. Ser. No. [Attorney Docket No. PF-0663 P, filed January 21, 1999], are hereby expressly incorporated by reference.

45 EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized

5 and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

10 5 Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was
15 isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

An alternative method of library construction was as follows:

20 The frozen tissue was homogenized and lysed in guanidinium isothiocyanate solution using a Brinkmann Homogenizer Polytron-PT 3000 (Brinkmann Instruments, Westbury NY). RNA was isolated as per Stratagene's RNA isolation protocol (Stratagene, La Jolla CA). RNA was extracted
15 twice with acid phenol, precipitated with sodium acetate and ethanol, resuspended in RNase-free water, and treated with DNase. Poly (A+) RNA was isolated using the OLIGOTEX kit (QIAGEN Inc, Chatsworth CA).

30 Poly(A+) RNA was used for cDNA synthesis and construction of the cDNA library according to the recommended protocols in the SUPERScript plasmid system (Life Technologies). The cDNAs were fractionated on a SEPHAROSE CL4B column (Amersham Pharmacia Biotech), and those cDNAs exceeding 400 bp were ligated into pINCY (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into DH5 α competent cells or ELECTROMAX cells (Life Technologies).

35 In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA
25 libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, *supra*, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the
40 appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), or pINCY (Incyte
45 Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli*
50

5 cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 α , DH10B, or
ElectroMAX DH10B from Life Technologies.

10 IL Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision using the UNIZAP vector system
15 (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or
WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge
Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8
Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN.
20 Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or
without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a
25 high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal
cycling steps were carried out in a single reaction mixture. Samples were processed and stored in
384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically
30 using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence
scanner (Labsystems Oy, Helsinki, Finland).

35 III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput
instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200
40 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or
the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared
using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as
the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).
45 Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides
were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the
ABI PRISM 373 or 377 sequencing system (Perkin-Elmer) in conjunction with standard ABI
protocols and base calling software; or other sequence analysis systems known in the art. Reading
50 frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel,
1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques
disclosed in Example V.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed
45 using a combination of software programs which utilize algorithms well known to those skilled in the
art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable
descriptions, references, and threshold parameters. The first column of Table 5 shows the tools,
55 35 programs, and algorithms used, the second column provides brief descriptions thereof, the third

column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:4-6. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel, 1995, *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of

5 the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

100

10 5 The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

15 10 The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding HPPL occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

30 V. Extension of HPPL Encoding Polynucleotides

20 20 The full length nucleic acid sequences of SEQ ID NO:4-6 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

40 Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

30 30 High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg²⁺, (NH₄)₂SO₄, and β-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C,

2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 µl to 10 µl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:4-6 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:4-6 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments.

Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μ Ci of [γ - 32 P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, *supra*.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al.

(1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

VIII. Complementary Polynucleotides

Sequences complementary to the HPPL-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HPPL. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of HPPL. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HPPL-encoding transcript.

IX. Expression of HPPL

Expression and purification of HPPL is achieved using bacterial or virus-based expression systems. For expression of HPPL in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express HPPL upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of HPPL in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding HPPL by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, HPPL is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham

Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from HPPL at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, *supra*, ch. 10 and 16). Purified HPPL obtained by these methods can be used directly in the following activity assay.

X. Demonstration of HPPL Activity

HPPL activity can be demonstrated by an *in vitro* hydrolysis assay with vesicles containing 1-palmitoyl-2-[1-¹⁴C]oleoyl phosphatidylcholine (Sigma-Aldrich). HPPL phospholipase A₂ activity is demonstrated by analysis of the cleavage products isolated from the hydrolysis reaction mixture.

Vesicles containing 1-palmitoyl-2-[1-¹⁴C]oleoyl phosphatidylcholine (Amersham Pharmacia Biotech.) are prepared by mixing 2.0 µCi of the radiolabeled phospholipid with 12.5 mg of unlabeled 1-palmitoyl-2-oleoyl phosphatidylcholine and drying the mixture under N₂. 2.5 ml of 150 mM Tris-HCl, pH 7.5, is added, and the mixture is sonicated and centrifuged. The supernatant may be stored at 4 °C. The final reaction mixtures contain 0.25 ml of Hanks buffered salt solution supplemented with 2.0 mM taurochenodeoxycholate, 1.0% bovine serum albumin, 1.0 mM CaCl₂, pH 7.4, 150 µg of 1-palmitoyl-2-[1-¹⁴C]oleoyl phosphatidylcholine vesicles, and various amount of HPPL diluted in PBS. After incubation for 30 min at 37 °C, 20 µg each of lyso-phosphatidylcholine and oleic acid are added as carriers and each sample is extracted for total lipids. The lipids are separated by thin layer chromatography using a two solvent system of chloroform:methanol:acetic acid:water (65:35:8:4) until the solvent front is halfway up the plate. The process is then continued with hexane:ether:acetic acid (86:16:1) until the solvent front is at the top of the plate. The lipid-containing areas are visualized with I₂ vapor; the spots are scraped, and their radioactivity is determined by scintillation counting. The amount of radioactivity released as fatty acids will increase as a greater amount of HPPL is added to the assay mixture while the amount of radioactivity released as lyso-phosphatidylcholine will remain low. This demonstrates that HPPL cleaves at the *sn*-2 and not the *sn*-1 position, as characteristic of phospholipase A₂ activity.

Alternatively, HPPL activity is measured by the hydrolysis of a fatty acyl residue at the *sn*-1 position of phosphatidylserine. HPPL is combined with the Tritium [³H] labeled substrate phosphatidylserine at stoichiometric quantities in a suitable buffer. Following an appropriate incubation time, the hydrolyzed reaction products are separated from the substrates by chromatographic methods. The amount of acylglycerophosphoserine produced is measured by counting tritiated product with the help of a scintillation counter. Various control groups are set up to account for background noise and unincorporated substrate. The final counts represent the tritiated

5 enzyme product [3H]-acylglycerophosphoserine, which is directly proportional to the activity of HPPL in biological samples.

XL. Functional Assays

10 HPPL function is assessed by expressing the sequences encoding HPPL at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10 μ g of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome 15 formulations or electroporation. 1-2 μ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics- 20 based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light 25 scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

30 The influence of HPPL on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding HPPL and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success 35 NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding HPPL and other genes of interest can be analyzed by northern analysis or microarray techniques.

XII. Production of HPPL Specific Antibodies

HPPL substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HPPL amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, *supra*, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, *supra*.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-HPPL activity by, for example, binding the peptide or HPPL to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIII. Purification of Naturally Occurring HPPL Using Specific Antibodies

Naturally occurring or recombinant HPPL is substantially purified by immunoaffinity chromatography using antibodies specific for HPPL. An immunoaffinity column is constructed by covalently coupling anti-HPPL antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HPPL are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HPPL (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HPPL binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HPPL is collected.

XIV. Identification of Molecules Which Interact with HPPL

HPPL, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HPPL, washed, and any wells with labeled HPPL complex are assayed. Data obtained using different concentrations of HPPL are used to calculate values for the number, affinity, and association of HPPL with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention

5 will be apparent to those skilled in the art without departing from the scope and spirit of the invention.

Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

Indeed, various modifications of the described modes for carrying out the invention which are

10 5 obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

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Table 1

Polypeptide SEQ ID NO:	Polynucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	4	2641779	LUNGTUT08	2641779H1 (LUNGTUT08), 3980344H1 (LUNGTUT08), 2203422F6 (SPINFET02), 701224H1 (SYNORAT03)
2	5	1430683	SINTBST01	1430683H1 (SINTBST01), 1430683F6 (SINTBST01), 4591542H1 (MASTTX01), 1654109X12 (PROSTUT08), 836234R6 (PROSNOT07), 1511418F6 (LUNGNOT14), 1513169F6 (PANCUTUT01)
3	6	1316804	BLADTUT02	1316804H1 (BLADTUT02), 1316804T6 (BLADTUT02), 2965020H1 (SCORNOT04), 3539795H1 (SEMVN0T04), SBFA00393F1, SBFA01391F1, SBFA03702F1, SBFA04815F1, SBFA04928F1

Table 2

Poly-peptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential glycosylation sites	Signature Sequences, Motifs and Domains	Homologous Sequences	Analytical Methods & Databases
1	145	S102 T33 S98	N89	Signal peptide: M1-I16; M1-G21 Active site histidine region: Y44-Q92 Active site aspartic acid region: Y88-G140 phospholipase A2 active site signature: G21-C145	Group IID secreted phospholipase A2, group II (g5359708) <u>Mus musculus</u> Group II Phospholipase A-2 (g204319) <u>Rattus norvegicus</u>	PROFILERSCAN HMMER-PFAM HMMER BLIMPS-BLOCKS BLIMPS-PRINTS SPSCAN BLAST-GenBank
2	605	S72 S78 S186 T282 S293 T430 T440 T506 T518 T540 S552 S566 S104 T397 T426	N456 N550		Cytosolic phospholipase A2 beta (g3811347) <u>Homo sapiens</u> Cytosolic phospholipase A2 (g508625) <u>Gallus gallus</u>	BLAST-GenBank

Table 2 cont.

Poly-peptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential glycosylation sites	Signature Sequences, Motifs and Domains	Homologous Sequences	Analytical Methods & Databases
3	456	S23 S99 T187 S284 S369 S404	N79 N365	Signal peptide: M1-G25; M1-A27 Lipase serine active site: I160 Triglyceride lipase GXSG (SEQ ID NO:10) consensus pattern: G164-G168 Putative lipase signature: T47-C245; C258-C296 Triacylglycerol lipase family signature: D72-F91; I117-A132; S159-Q177; C258- N273	Serine phospholipid- specific phospholipase A (g1817556) <u>Rattus norvegicus</u>	HMME-PFAM BLIMPS-PRINTS HMME SPSCAN BLAST-GenBank MOTIFS

Table 3

Nucleotide SEQ ID NO:	Selected Pragment(s)	Tissue Expression	Disease or Condition (Fraction of Total)	Vector
4	14-73	Reproductive Cardiovascular	Cancer and proliferating tissue (0.890) Inflammation/Trauma (0.220)	pINCY
5	95-154	Reproductive Nervous	Cancer and proliferating tissue (0.720) Immune Response (0.240)	PBLUESCRIPT
6	1148-1192	Reproductive Gastrointestinal	Cancer (0.500) Inflammation and Immune Response (0.420)	pINCY

Table 4

Nucleotide SEQ ID NO:	Library	Library Description
1	LUNGTUT08	Library was constructed using RNA isolated from lung tumor tissue removed from a 63-year old Caucasian male during a right upper lobectomy with fiberoptic bronchoscopy. Pathology indicated a grade 3 adenocarcinoma. Patient history included rectal cancer and tobacco abuse. Family history included stomach cancer and lung cancer.
2	SINTBST01	Library was constructed using RNA isolated from ileum tissue obtained from an 18-year old Caucasian female removed during bowel anastomosis. Pathology indicated Crohn's disease of the ileum, involving 15 cm of the small bowel.
3	BLADTUT02	Library was constructed using RNA isolated from bladder tumor tissue removed from an 80-year-old Caucasian female during a radical cystectomy and lymph node excision. Pathology indicated grade 3 invasive transitional cell carcinoma. Family history included acute renal failure, osteoarthritis, and atherosclerosis.

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, ifasta, fastx, ifastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value= 1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A Blocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Atwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and, if applicable, Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits for PFAM hits, depending on individual protein families

Table 5 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Normalized quality score \geq GCG-specified "HIGH" value for that particular Prosite motif. Generally, score = 1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score = 120 or greater; Match length = 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score = 3.5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. <u>supra</u> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

Claims

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What is claimed is:

1. A substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:3 and fragments thereof.
2. A substantially purified variant having at least 90% amino acid sequence identity to the amino acid sequence of claim 1.
3. An isolated and purified polynucleotide encoding the polypeptide of claim 1.
4. An isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide of claim 3.
5. An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of claim 3.
6. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide of claim 3.
7. A method for detecting a polynucleotide, the method comprising the steps of:
 - (a) hybridizing the polynucleotide of claim 6 to at least one nucleic acid in a sample, thereby forming a hybridization complex; and
 - (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of the polynucleotide in the sample.
8. The method of claim 7 further comprising amplifying the polynucleotide prior to hybridization.
9. An isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5 and fragments thereof.
10. An isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide of claim 9.
11. An isolated and purified polynucleotide having a sequence which is complementary

5 to the polynucleotide of claim 9.

12. An expression vector comprising at least a fragment of the polynucleotide of claim 3.

10 5 13. A host cell comprising the expression vector of claim 12.

14. A method for producing a polypeptide, the method comprising the steps of:

15 a) culturing the host cell of claim 13 under conditions suitable for the expression of the polypeptide; and

10 b) recovering the polypeptide from the host cell culture.

20 15. A pharmaceutical composition comprising the polypeptide of claim 1 in conjunction with a suitable pharmaceutical carrier.

15 16. A purified antibody which specifically binds to the polypeptide of claim 1.

25 17. A purified agonist of the polypeptide of claim 1.

30 20 18. A purified antagonist of the polypeptide of claim 1.

19. A method for treating or preventing a disorder associated with decreased expression or activity of HPPL, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 15.

25 20. A method for treating or preventing a disorder associated with increased expression or activity of HPPL, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 18.

1 MELALLCGLVVMA - GVIPIQGGI LNLNKMV 2641779
1 MKVLLLLAVVIMAF GS IQVQGS LLEFGQM I GI 204319

30 KQVTGKMPILSYWPY GCHCG LGGRG QPKDA 2641779
31 LFKTGKRRADVSYGFY GCHCG VGGRG S PKDA GI 204319

60 TDWCCQTHDCCYDHLKTQGGCIYKDYRYN 2641779
61 TDWCCVTHDCCYNRL EKRGGCTKFLT YKFS GI 204319

90 FSQGNIHCS DKGSWC EQQLCA CDKEVA FCL 2641779
91 YRGGQISCS TNQDS CRKQLC QCDKAA AE CF GI 204319

120 KRNLDTYQKRLRFYWRPHCRGQT PGC 2641779
121 ARNKKSYSLKYQFY LNKFC KGT PSC GI 204319

FIGURE 1

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 1 M S F I D P Y Q H I V V E H Q Y S H V F T V T V - R K A T N G I 508625

 21 L T D T G L - - - - - L V L F C P A P - - C P - - 1430683
 30 V T K G A I G D M L D T P D P Y V E L F I P S A P D C R K R G I 508625

 37 - - - - - F F F F F E M - - - - - 1430683
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 44 - - - - - E S L S V A Q A G V Q W R D L G S L Q P 1430683
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 64 P P L G F K R F S C L S L P S S W D Y R L R E L A V R L G F 1430683
 120 V Q L T F N N V T E M T L E L S L E V C - S S T D L R F S M G I 508625

 94 G P C A E E Q A F L S R R K Q V V A A A L R Q A L Q L D G - 1430683
 149 A L C D E E K K F R Q Q R K D N I M Q S M K S F L G E E N S G I 508625

 123 - D L Q E D - E I P V V A I M A T G G G I R A M T S L Y G Q 1430683
 179 K N L T T S R D V P V I A V L G S G G G F R A M V G F A G V G I 508625

 151 L A G L K E L G L L D C V S Y I T G A S G S T W A L A N L Y 1430683
 209 M K A L Y E S G V L D C A T Y I A G L S G S T W Y M S T L Y G I 508625

 181 E D P E W S Q K D L A G P T E L L K T Q V T K N K L G V L A 1430683
 239 S H P D F P E K G P K E I N Q E L M N S V S H N P L L L T G I 508625

FIGURE 2A

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211 P S Q L Q R Y R Q E L A E R A R L G Y P S C F T N L W A - L 1430683
 269 P Q K V K R Y I E A L W N K K S S G Q P V T F T D I F G M L GI 508625

 240 I N E A L L H D E P H D H K L S D Q R E A L S H G Q N P L P 1430683
 299 I G E T L I H N - R M D T T L S D M K E K V S E A Q C A L P GI 508625

 270 I Y C A L N T K G Q S L T T F E F G E W C E F S P Y E V G F 1430683
 328 L F T C L H V K P D - V S E L M F A D W V E F S P Y E I G M GI 508625

 300 P K Y G A F I P S E L F G S E F F M G Q L M K R L P E S R I 1430683
 357 A K Y G T F M S P D L F G S K F F M G T V V K K Y S E N P L GI 508625

 330 C F L E G I W S N L Y A A N L Q D S L - - - - - Y 1430683
 387 H F L M G V G S A F S I L F N R V L G V S N S Q N K G P T GI 508625

 350 W A S E P S Q F W - D R W V R N Q A N L D K E Q V P L L K I 1430683
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 379 E E P P S T A - - - - - G R I A E F F T D L L T W R 1430683
 447 T E N S E A N E E Y Q N S S Q E S W V Q R M L M A L V G D S GI 508625

 400 P L - - - - - A Q A T H N F L R G L H F H K D Y F Q H P 1430683
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 507 L A D L L T Q E S V E E D E L D A A V A D P D E F E R I Y E GI 508625

FIGURE 2B

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445 - - - - - H L C L L D V G Y L I N T S C L P L L Q P T R 1430683
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 539 R T P E E A A A - G E V N L - S S S D S P Y H Y T K V T Y S 1430683
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 597 Q R R R Q R R P H 1430683
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 605
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FIGURE 2C

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FIGURE 3A

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271	LENSCPL	MAFP	CAS	YKAF	LAG	CLDC	FNPF	1316804		
271	LENT	CP	LM	AF	PC	AS	YKAF	LAG	CLDC	FNPF
301	LLSCPR	IG	LV	EQ	GV	KIE	PL	PK	EV	KVYLLT
301	LLSCPR	IG	LV	ER	GV	KIE	PL	PK	EV	RVYLLQ
331	TSSAPY	CM	HH	SL	VE	FL	KE	LR	NK	DTNIEVT
331	TSSAPY	CV	HH	SL	VE	FN	LE	KE	RR	KDTSIEVT
361	FLSSNI	TSS	KIT	IP	KQ	RY	GK	II	AH	ATP
361	FLGN	NV	TSS	VKIT	IP	KD	HL	EG	RI	AHQNP
391	QCQIN	QVK	FK	FQ	SS	NR	VW	KK	DR	TTIIGKFC
391	HCQIN	QVK	LKF	HI	SS	RV	WR	KD	RT	PIVGTFC
421	TALL	LP	VND	RE	KM	VC	LP	EP	VNL	QASVTVSCD
421	TAP	LP	VND	SK	KT	VC	IP	EP	VR	LQVSMALRD
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FIGURE 3B

SEQUENCE LISTING

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 HILLMAN, Jennifer L.
 BANDMAN, Olga
 GUEGLER, Karl J.
 CORLEY, Neil C.
 BAUGHN, Mariah R.
 AZIMZAI, Yalda
 LAL, Preeti
 LY, Dyung Aina M.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: C12N 15/55, A61K 38/46, C07K 16/40, C12N 9/20, C12N 15/63, C12Q 1/68	A3	(11) International Publication Number: WO 00/24911 (43) International Publication Date: 04 May 2000 (04.05.2000)
(21) International Application Number: PCT/US99/25021 (22) International Filing Date: 27 October 1999 (27.10.1999) (30) Priority Data: 09/181,317 27 October 1998 (27.10.1998) US 09/234,726 21 January 1999 (21.01.1999) US (60) Parent Application or Grant INCYTE PHARMACEUTICALS, INC. [/]; O. HILLMAN, Jennifer, L. [/]; O. BANDMAN, Olga [/]; O. GUEGLER, Karl, J. [/]; O. CORLEY, Neil, C. [/]; O. BAUGHN, Mariah, R. [/]; O. AZIMZAI, Yalda [/]; O. LAL, Preeti [/]; O. LU, Dyung, Aina, M. [/]; O. HILLMAN, Jennifer, L. [/]; O. BANDMAN, Olga [/]; O. GUEGLER, Karl, J. [/]; O. CORLEY, Neil, C. [/]; O. BAUGHN, Mariah, R. [/]; O. AZIMZAI, Yalda [/]; O. LAL, Preeti [/]; O. LU, Dyung, Aina, M. [/]; O. BILLINGS, Lucy, J.; O.		Published
(54) Title: HUMAN PHOSPHOLIPASES (54) Titre: PHOSPHOLIPASES HUMAINES (57) Abstract The invention provides human phospholipases (HPPL) and polynucleotides which identify and encode HPPL. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HPPL. (57) Abrégé L'invention concerne des phospholipases humaines (HPPL) et des polynucléotides qui identifient et codent les HPPL. L'invention concerne aussi des vecteurs d'expression, des cellules hôtes, des anticorps, des agonistes et des antagonistes. L'invention concerne enfin des procédés pour diagnostiquer, traiter ou prévenir les troubles liés à l'expression de HPPL.		

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(51) International Patent Classification ⁷ : C12N 15/55, 9/20, C12Q 1/68, C12N 15/63, A61K 38/46, C07K 16/40		A3	(11) International Publication Number: WO 00/24911
			(43) International Publication Date: 4 May 2000 (04.05.00)
(21) International Application Number: PCT/US99/25021		(72) Inventors; and (75) Inventors/Applicants (for US only): HILLMAN, Jennifer, L. [US/US]; 230 Monroe Drive, #12, Mountain View, CA 94040 (US). BANDMAN, Olga [US/US]; 366 Anna Avenue, Mountain View, CA 94043 (US). GUEGLER, Karl, J. [CH/US]; 1048 Oakland Avenue, Menlo Park, CA 94025 (US). CORLEY, Neil, C. [US/US]; 1240 Dale Avenue, #30, Mountain View, CA 94040 (US). BAUGHN, Mariah, R. [US/US]; 14244 Santiago Road, San Leandro, CA 94577 (US). AZIMZAI, Yalda [US/US]; 2045 Rock Springs Drive, Hayward, CA 94545 (US). LAL, Preeti [IN/US]; 2382 Lass Drive, Santa Clara, CA 95054 (US). LU, Dyung, Aina, M. [US/US]; 55 Park Belmont Park, San Jose, CA 95136 (US).	
(22) International Filing Date: 27 October 1999 (27.10.99)			
(30) Priority Data: 09/181,317 27 October 1998 (27.10.98) US Not furnished 27 October 1998 (27.10.98) US 09/234,726 21 January 1999 (21.01.99) US Not furnished 21 January 1999 (21.01.99) US			
(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications US 09/181,317 (CIP) Filed on 27 October 1998 (27.10.98) US Not furnished (CIP) Filed on 27 October 1998 (27.10.98) US 09/234,726 (CIP) Filed on 21 January 1999 (21.01.99) US Not furnished (CIP) Filed on 21 January 1999 (21.01.99)		(74) Agents: BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).	
(71) Applicant (for all designated States except US): INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
		Published With international search report.	
		(88) Date of publication of the international search report: 10 August 2000 (10.08.00)	
(54) Title: HUMAN PHOSPHOLIPASES			
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(57) Abstract			
The invention provides human phospholipases (HPPL) and polynucleotides which identify and encode HPPL. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HPPL.			

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CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 99/25021

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12N15/55 C12N9/20 C12Q1/68 C12N15/63 A61K38/46 C07K16/40		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N C12Q A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Emest3 Database Entry Aa762051 Accession number AA762051; 28 January 1998 MARRA M. ET AL.: "The WashU-HHMI Mouse EST Project" XP002130208 the whole document	2-13
A	US 5 279 957 A (RICHARD GROSS) 18 January 1994 (1994-01-18) the whole document	1-16,19
A	WO 95 02328 A (INDIANA UNIVERSITY FOUNDATION) 26 January 1995 (1995-01-26) the whole document	1-16,19
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "Z" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
10 February 2000		16.05.2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentean 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer MONTERO LOPEZ B.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/25821

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>JUN ISHIZAKI ET AL.: "Cloning and characterization of novel mouse and human secretory phospholipase A2s" JOURNAL OF BIOLOGICAL CHEMISTRY, [Online] vol. 274, no. 35, 27 August 1999 (1999-08-27), pages 24973-24979, XP002130207 MD US Retrieved from the Internet: <URL:http://intl.jbc.org/cgi/content/full/ 274/35/24973> [retrieved on 2000-02-08] the whole document -----</p>	1-16,19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/25021

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 19 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.
2. ☒ Claims Nos.: 17, 18, 20
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20 partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17, 18, 20

Present claims 17, 18 and 20 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is not to be found, however, for any specific example of the compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, no search has been carried out for claims 17, 18 and 20.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-20 partially

Polypeptide of SEQ ID NO:1, fragments and variants thereof; polynucleotide encoding it of SEQ ID NO:4 and variants thereof; use in a hybridizing method; vector and host cell containing the polynucleotide; use thereof for producing the polypeptide; pharmaceutical composition comprising the polypeptide; antibody, agonist and antagonist to the polypeptide and therapeutic use of the antagonist.

2. Claims: 1-8 partially, 12-20 partially

Polypeptide of SEQ ID NO:3, fragments and variants thereof; polynucleotide encoding the same and variants thereof; use in a hybridizing method; vector and host cell containing the polynucleotide; use thereof for producing the polypeptide; pharmaceutical composition comprising the polypeptide; antibody, agonist and antagonist to the polypeptide and therapeutic use of the antagonist

3. Claims: 9-11 partially

Polynucleotide comprising SEQ ID NO:5 and fragments thereof; variant having at least 90% identity and polynucleotide complementary to it.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 99/25021

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5279957 A	18-01-1994	NONE	
WO 9502328 A	26-01-1995	AU 7362294 A CA 2167296 A US 5972677 A	13-02-1995 26-01-1995 26-10-1999